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Contents

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THE SPORES OF *HISTOPLASMA*[1]

BY ELEANOR SILVER DOWDING[2]

Abstract

Tuberculate spores distinguish the mold stage of *Histoplasma* from that of related pathogenic fungi. The tuberculations are not thickenings of the spore wall. They are extensions of the spore contents through the wall openings. The whole or the tip of a tuberculation may become detached and act as a spore. The yeastlike pathogenic phase of *Histoplasma* originates either from the hyphae, from the small spores (conidia), or from the tuberculations of the large spores (chlamydospores). *Histoplasma* can be recovered in culture from the lungs of white mice that have inhaled the spores. No chlamydospores have been discovered in sections of their lungs. Nevertheless, in districts of high histoplasmin sensitivity, when lungs with nodules are examined in routine autopsies, the finding of any spherical body with external spores might well suggest an arrested histoplasmosis.

Introduction

Formerly histoplasmosis was regarded as a regularly fatal disease. We suspect today that it also produces nonfatal respiratory conditions (14). Similarly, coccidioidomycosis was known earlier in its grave disseminated form and only later in its milder respiratory manifestations.

That *Coccidioidis immitis* is responsible for an influenzalike disease rests not alone upon indirect evidence, i.e., that many persons in the Southwest United States with lung calcifications are coccidioidin-sensitive (1). It also rests upon the following direct observations.

(1). Out of 3000 routine autopsies in San Francisco, Cox and Smith (5) found four lungs with hard nodules. The nodules contained spherules 15 to 40μ in diameter. One spherule, which had spores within it, was identical in size and structure with the fungus *Coccidioides immitis*. The spherules in the other lungs were probably also *C. immitis*. These bodies were found in persons who died of causes other than fungous infection.

(2). Many natives of California acquire San Joaquin fever and erythema nodosum. Dickson and Gifford (6) showed not only that these persons possess lung calcifications and that they are sensitive to the fungus extract coccidioidin, but also that the sputa and stomach washings of many of them contain spores of *C. immitis*.

In fact it is now proved that *C. immitis* produces a benign infection.

That *Histoplasma capsulatum* causes benign infection rests only upon indirect evidence, i.e., that many persons in East Central United States are

[1] *Manuscript received June 3, 1948.*
Contribution from the Provincial Laboratory, University of Alberta, Edmonton, Alta.
[2] *Medical Mycologist.*

histoplasmin-sensitive (13). No spores of *Histoplasma* have yet been found in the lungs, sputum, or stomach washings of histoplasmin-sensitive persons.

It was thought, therefore, that a more intimate knowledge of the spores of *Histoplasma* in their saprophytic mold phase and in their transition to the parasitic yeast phase might be a step toward a better understanding of histoplasmosis.

Methods

For the microscopic study of spores, the usual method of transferring the mycelium by means of an inoculating loop to the mounting medium disarranges the hyphae and scatters the spores of *Histoplasma*. Therefore, the following method was employed.

A sterile cover slip was lowered over a Petri dish culture until it came into contact with the aerial mycelium. For immediate examination, the cover slip, with its adherent spores, was placed on a slide containing a drop of cotton blue and lactic acid (Fig. 1, *c*). For cultural study, it was sealed with vaseline upon a hollow-ground slide containing water, with or without the addition to the spore print of nutrient broth or agar (Fig. 1, *a* and *b*). The microcultures so made were left either at room temperature or at 37° C. for about a week. Sometimes individual spores were kept under observation under the microscope for several days and drawn with the camera lucida at intervals.

When a cover slip is brought into contact with a *Histoplasma* culture by the method described above, a deposit of spores and a mosaic of hyphae adhere to it. Most of the hyphae are dead and only faintly visible, but many of the conidia are still arranged on them in the original position (Fig. 1, *d*). Chlamydospores appear under transmitted light as dark globules because they are immersed in liquid drops (Fig. 1, *b*). On lowering the cover slip upon mounting medium, the drops on the chlamydospores are pressed out into smears containing conidia (Fig. 1, *c* and *d* and Fig. 3, *c*). When a microculture has remained for some days in water the mosaic of hyphal remnants disappears, leaving behind a network of mucilaginous-appearing strands (Fig. 2, *ad*).

In the network resulting from the disorganized mycelium were sometimes found rectangular, doubly-refringent bodies with re-entrant angles (Fig. 2, *ad*). These were similar in every respect to the 'mosaic fungus' frequently seen in epidermal scales from ringworm lesions. They are additional evidence that such mosaic structures are indeed disintegration products of fungous hyphae, as has been previously suggested from this laboratory (8).

When water or broth was added to the spore deposit and the microculture so made was kept under observation on the stage of the microscope, it was found that conidia and small hyphal fragments sometimes moved towards the chlamydospores. This agglutination phenomenon, although apparently of no biological significance, is a hindrance in the laboratory because it tends to obscure the origin of the conidia. Thus the appearance of a conidium close to the chlamydospores such as the one in Fig. 3, *e* (an arrow points to it)

leaves some doubt as to whether it arose from the tuberculation or from a neighboring hypha. Furthermore, by the method just described few of the spores ever became truly wet. In fact many of them remained in an air

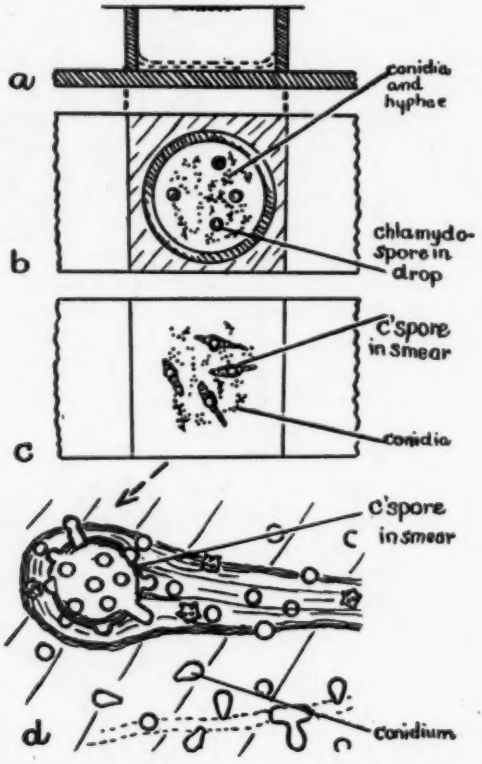


FIG. 1. *Diagrams of spore-deposit preparations. a, Van Tieghem cell, side view. b, The same from above, showing spores and hyphae. The chlamydospores are in liquid drops. c, The cover slip shown in a placed upon a slide bearing a drop of mounting fluid. The drops shown in b are spread out into streaks. d, Part of preparation c at higher magnification.*

bubble in the nutrient medium. For the study of the yeastlike phase particularly, it was necessary to insure (1) that the spores could not agglutinate, (2) that they became wet. The following method was used to bring this about.

By means of a pipette, the depression in a hollow-ground slide was almost filled with Sabouraud's medium. When the agar had solidified, a drop of plain broth was placed on top of it. The depression was then rimmed with vaseline. The cover slip, bearing its imprint of spores, was lowered, spores down, over the medium. The preparation was cautiously warmed, only enough to melt the vaseline. The cover slip then settled down on the moist

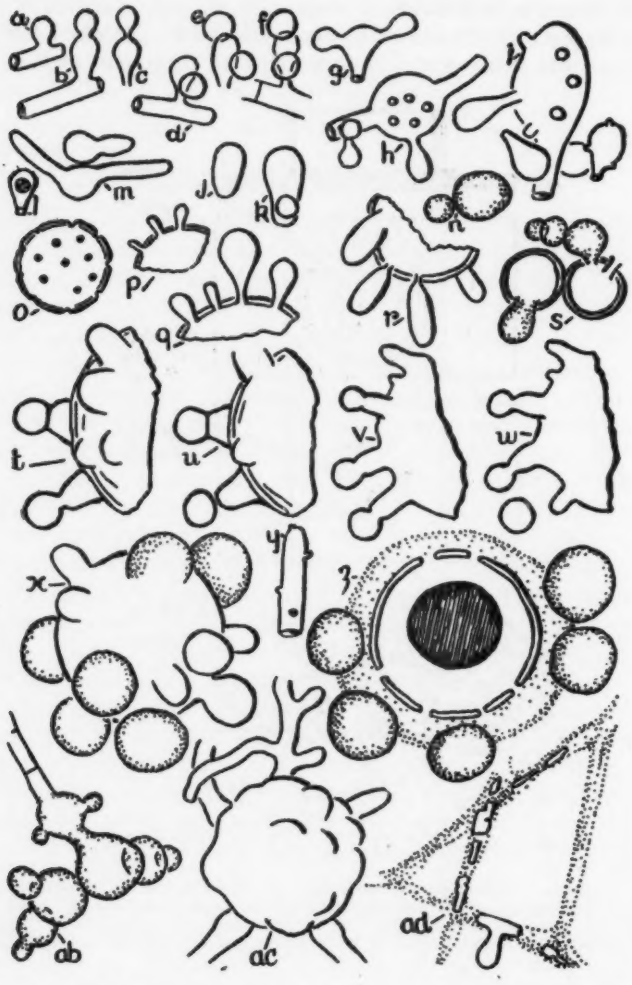


FIG. 2. *Spores (all of H. capsulatum but r and s). a to m, Conidia: b to k, secondary conidia; j, conidium; k, same drawn one hour later; l and m, stages in germination. n, Yeast cell with one bud. o to q, Development of a chlamydospore. r, H. parvum, atypical chlamydospore. s, B. dermatitidis, budding chlamydospore. t, Part of a chlamydospore in moist air. It is partly covered by mucilage but the tuberculations are exposed. An upper one is septate, a lower one, constricted. u, The same spore 13 hr. later. The lower tuberculation has cut off a conidium. v and w, Similar stages in another chlamydospore. x, Incubated chlamydospore with tuberculations some of which have been converted into yeast cells. y, Hypha with minute wall thickenings; z, Later stage of x showing yeast cells in a mucilaginous envelope surrounding chlamydospore. The darkly shaded body is an oil globule within the spore. ab, The yeast cells developing from hypha. ac, Germinating chlamydospore. ad, Disorganized aerial hyphae with one conidium and mosaic of disintegration products. Magnification: 1200.*

agar in such a way that no air bubbles broke the contact between the cover slip and medium. The film of broth became absorbed by the agar into which the spores settled. The preparation was put on a piece of bent glass tubing that was placed on a damp filter paper lying in the bottom of a Petri dish. The dish was kept for a week in an incubator set at 37° C.

The Spores

The spores of the mold phase of *Histoplasma capsulatum* have been described by Conant (2) essentially as follows: conidia are spherical to pyriform, smooth-walled, 2.5μ in diameter, either sessile upon the hyphae or borne on short lateral branches. The chlamydospores are at first smooth-walled but later show tuberculate sculpturings, and vary from the size of the conidia up to 15μ, the largest being covered with fingerlike protuberances sometimes 6μ long. The conidia and chlamydospores contain fat droplets.

It was found that not all strains of *Histoplasma* examined in this Laboratory produced spores. However, one strain, satisfactory in this respect, was obtained through the kindness of Dr. C. W. Emmons from the United States Public Health Service, and was used in the present investigation. The strain was similar to that described by Conant. Sometimes the spores and hyphae were not smooth but slightly rough (Fig. 2, *y*; Fig. 3, *a*).

When the spores of *Histoplasma* are in air their walls appear irregular and their contents are not visible, but in liquid the walls appear regular and the oil globules within show clearly (Fig. 2, *l* and *z*). The spores are difficult to wet. The chlamydospores, particularly, usually remain dry even when mounted in water or broth, a behavior due probably to the irregularity of their shape and to the secretion that envelops them. In Fig. 3, *a*, most of the spores are dry, in Fig. 4, *f* they are wet, and the matrix in which they are embedded is drawn out into mucilaginous strings.

Conidia

The conidia of *Histoplasma* are borne laterally upon the hyphae. Most of these hyphae are devoid of contents and their walls are only faintly visible (Fig. 3, *a*). On living hyphae, no scars or collarettes have ever been seen that might suggest that spores have been detached from them. It would seem that, usually at least, the conidia are set free by the disintegration of the aerial mycelium.

A conidium is capable of producing one or several secondary conidia as is shown in Fig. 2, *b* to *i*. A single conidium was kept under observation for two days and it was observed to produce a secondary spore during an interval of one hour (Fig. 2, *j* and *k*).

The distinction between conidium and chlamydospore is not clear-cut. Spherical hyphal swellings intermediate in size between them are often encountered. These also are capable of producing one to several secondary conidia (Fig. 2, *h* and *i*).

When a conidium is planted in broth and left for four days at room temperature it enlarges and grows out into one or two germ tubes (Fig. 2, *m*), which develop into a mycelium and repeat the saprophytic mold phase.

The mold stage may be converted to the yeast stage by the method previously described. A conidium planted in agar and left for three days at 37° C., enlarges considerably and forms a thin-walled yeast cell with granular contents (Fig. 2, *n*). Fig. 4, *b* shows conidia (with oil drops) and yeast cells. The yeast cell then either produces a short germ tube (pseudomycelium) or one to several buds (Fig. 4, *a*). These buds are widely variable in size, some of them being as small as the cells found in infected animal tissue and others measuring 7μ (Fig. 4, *a* and *f*).

Chlamydospores

In two-weeks-old plate cultures of *Histoplasma*, chlamydospores are to be found among the younger peripheral hyphae. They may be intercalary or terminal. At this age they are smooth and thin-walled. Later, thickening is deposited unevenly upon the walls in such a way as to leave circular pits (Fig. 2, *o*). From the pits, fingerlike projections (tuberculations) extend.

The tuberculations are usually cylindrical, 2 to 3μ in diameter, and sometimes constricted near the tips (Fig. 3, *b* and *d*). The terminal knobs are white, while the rest of the spore wall is brown (Fig. 3, *b* and *d*).

In cotton blue – lactic acid solution, the spore contents stain blue and plasmolyze slightly. In this medium, spore contents can be seen to extend in narrow strands from the spore itself through the wall openings into the tuberculations. *Therefore we may conclude that the tuberculations are not wall thickenings but that they are hollow and that their contents are continuous with that of the spore itself.*

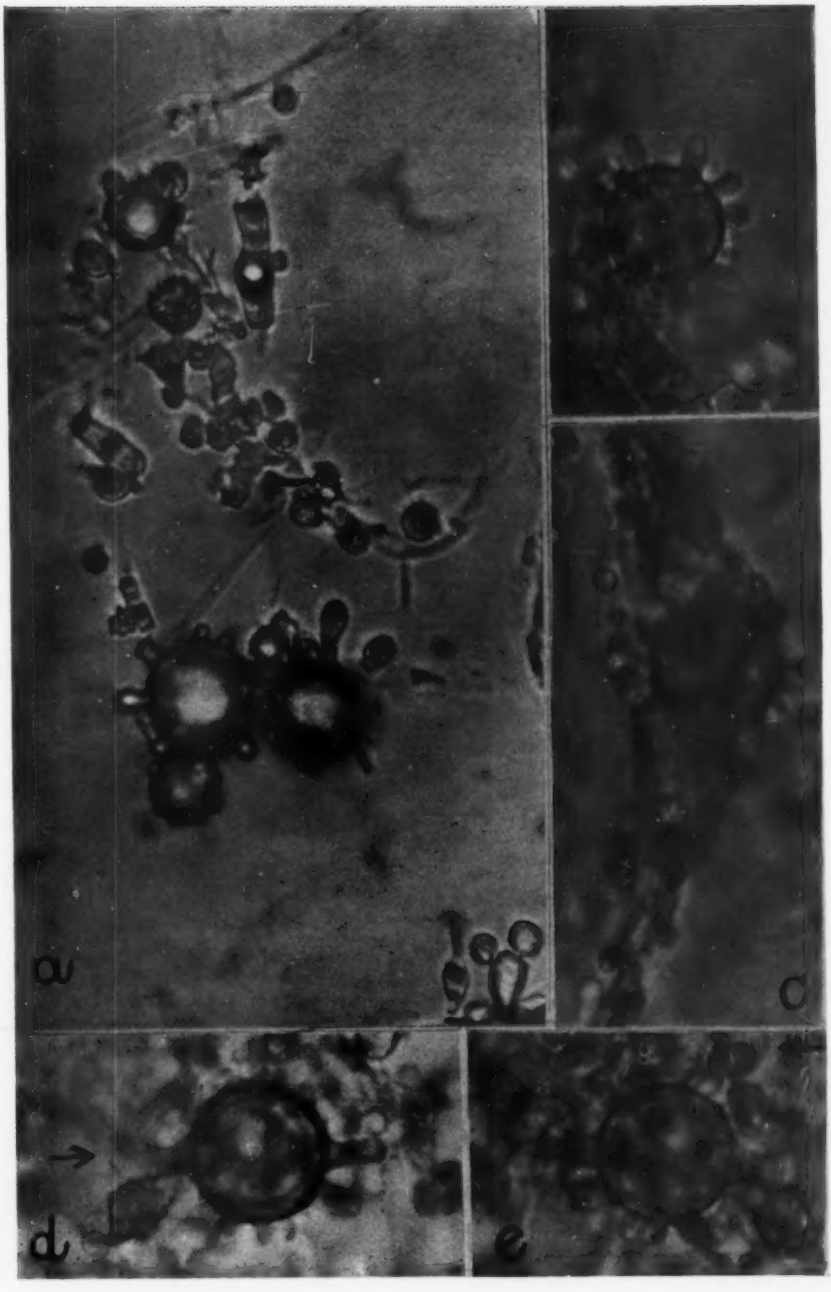
Chlamydospores that were not yet tuberculate were planted in broth and left for a week at room temperature. They germinated (Fig. 2, *ac*) and developed into a new mycelium, repeating the saprophytic phase.

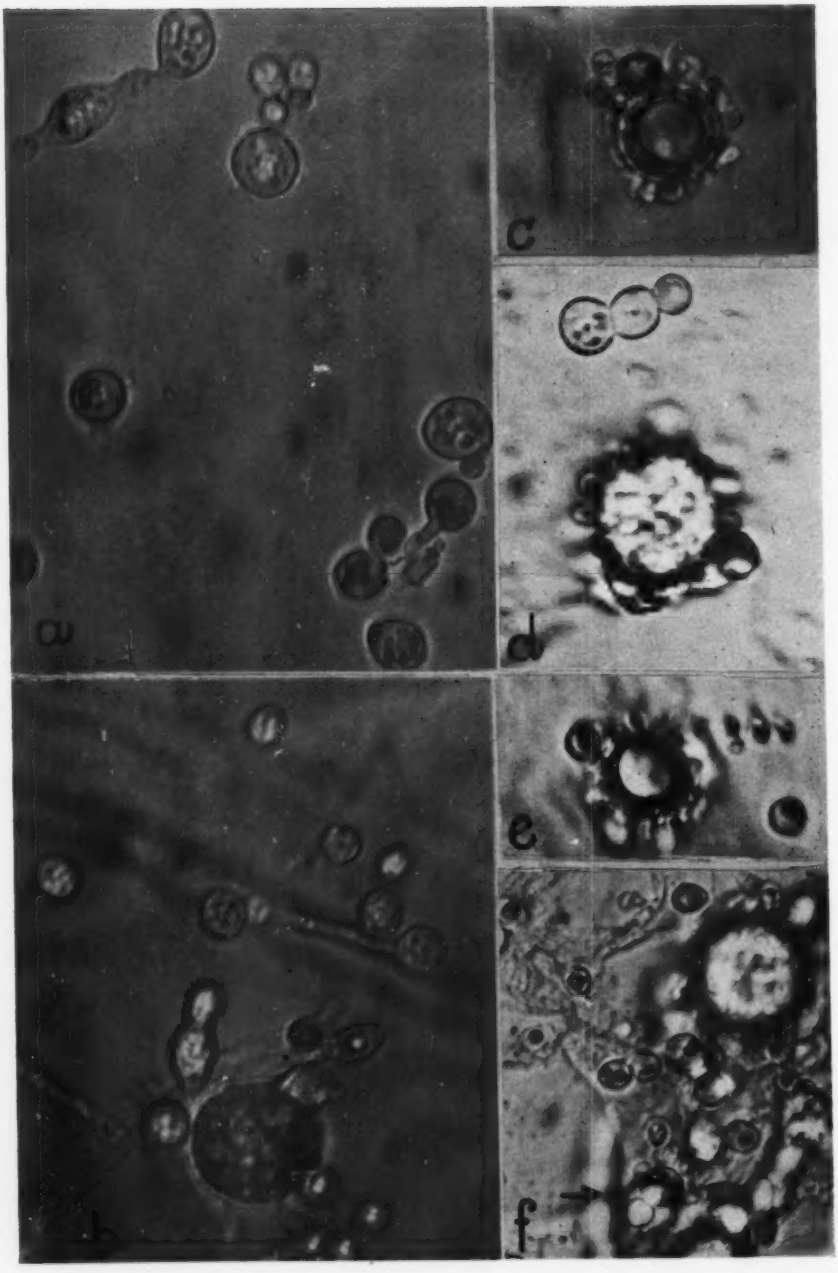
Tuberculate chlamydospores were left at room temperature in moist air and watched at successive intervals under the microscope. During an interval of 13 hr. it was found that tips of the tuberculations of two of them had become free from the spore (Fig. 2, *t* to *w*). It therefore appears that chlamydospores, like conidia, may produce secondary spores.

Tuberculate chlamydospores were left at 37° C. in agar using the method described earlier in the paper. After a day or two it was found that the tuberculations were no longer cylindrical but were pear-shaped or spherical,

FIG. 3. *Spores developed in moist air. a, Deposit composed of mycelium most of it dead and only faintly visible; conidia, dry ones showing rough wall, wet ones showing oil globule within, one at lower right producing secondary conidia; chlamydospores, one to left with cylindrical tuberculations, one to right, later stage, with two conidia. b, c, d, and e, Chlamydospores that have been mounted in cotton blue. c, Mucilaginous smear with embedded spores probably originated from the chlamydospore. d, The tuberculations at the right with two young conidia arising as colorless spherical knobs, to the left faintly visible, a larger one. e, Older stages of d, at upper right a mature secondary conidium. Magnification: 1500.*

PLATE I




a
b
c
d
e
f

of the same size and shape as conidia and, like them, each contained a central globule (Fig. 4, *d*). We may safely say that they *are* secondary conidia.

After three days in the incubator, some of the secondary conidia begin to enlarge considerably. They are now thin-walled oval or spherical yeast cells (Fig. 2, *x* and Fig. 4, *c*, *d*, and *e*), indistinguishable from those arising from hyphae or conidia.

Fig. 2, *z* shows the final stage of the incubated chlamydospore. It is now devoid of tuberculations. Openings in the spore wall are all that remains to mark their place. The spore is empty except for a single oil globule and is surrounded by a mucilaginous sheath containing yeast cells. The reader's attention is directed to the remarkable similarity between this spore and a spore of *Blastomyces brasiliensis* Conant and Howell. Such a spore within infected tissue is shown in Fonseca and Almeida's photograph reproduced by Moore (12) (Fig. 6, *f*), and can be compared with the photograph of *H. capsulatum* to the left of it.

Three Genera Compared

Sometimes young cultures of *Histoplasma capsulatum*, *Haplosporangium parvum*, and *Blastomyces dermatitidis* possess a white tufted or prickly center and a mucoid periphery, and are all strongly similar. Sometimes strains of the three genera possess few if any spores to distinguish them. Their conidia, when they are produced, are not particularly diagnostic (Fig. 5, *b*, *d*, and *f*). Their chlamydospores are more distinctive, as can be seen from Fig. 5, *a*, *c*, and *e*.

The spores of *Haplosporangium* and of *Blastomyces*, which have been obtained in the laboratory by incubation, are known in animal tissue. The budding chlamydospores of *Histoplasma*, which the writer obtained by incubation, have not been described in animals. The only cells of *Histoplasma* known to pathologists are small intracellular oval ones 1 to 5μ in diameter (3). It is suggested that, in districts of high histoplasmin sensitivity, when lungs with nodules are examined in routine autopsies, the finding of any spherical body with external spores might be interpreted as a budding chlamydospore and might suggest an arrested histoplasmosis.

Histoplasma and Haplosporangium

Histoplasma capsulatum and Haplosporangium parvum, the mouse lung fungus, are similar in the following respects. (1). Both grow and produce spores when inoculated upon soil. (2). Their spores cannot be jarred loose easily

FIG. 4. *The spores developed in agar at 37° C. a, Cells producing buds and at the upper left, pseudomycelium. b, To the right of the chlamydospore, conidia containing single oil drops, to the lower left a hypha bearing two conidia that have been converted into yeast cells, one of them budding. c, Chlamydospore producing conidia, those at the upper left enlarged to yeast cells. d, As c (the conidia being on the upper left surface of the chlamydospore, the yeast cells on its lower right). At the top of the picture is a yeast cell with two buds. e, Chlamydospore similar to c. f, Conidia, some on chlamydospore and some on disintegrated hyphae. At the bottom a yeast cell with five buds. Magnification: 1500.*

from the mycelium; they are not air-borne. (3). The spores of both are adhesive and can be transferred by contact. (4). In nature both fungi infect wild rodents (7, 9, 10). Further similarity is shown in the experiments now to be described.

A suspension of *Histoplasma* spores in sterile water was dropped on to the nostrils of six white mice so that they inhaled the drops. After the nasal inoculations, the animals were killed at intervals, ranging from one day to three weeks. From the lungs of three of the mice, cultures were obtained, which, from the appearance of their conidia (Fig. 6, *b*), and also their tuberculate chlamydospores, were identified with certainty as *H. capsulatum*. When the lungs of the six animals were sectioned and stained, however, no fungous spores were discovered in them.

A similar experiment was carried out with *Haplosporangium parvum*, this time using six wild white-footed deer mice trapped near Red Deer, Alta. It had been previously determined that in a control group of 33 animals from the same area only one animal was found with naturally occurring *Haplosporangium* infection. The deer mice were killed at intervals between one to five months after nasal inoculation. From the lungs of three of the six animals were obtained cultures that were identified with certainty as *H. parvum*. Furthermore, sections of the lungs of the same three animals showed the large chlamydospores characteristic of *H. parvum*.

The above experiments go to show that rodents may inhale the spores of *Histoplasma* and of *Haplosporangium* into their lungs.

Haplosporangium parvum was first discovered in 1942 in the squirrels, pocket mice, and kangaroo rats of Arizona (9). Subsequently it was found in deer mice in Alberta (7). Later, an organism that was similar to, if not identical with, *H. parvum* was described from a rock rabbit in Montana (11). Dr. Johnstone of the Department of Parasitology of the University of California School of Medicine showed the author similar bodies in his sections of lungs of a California water rat. Recent personal communications from other observers suggest that the fungus may also infect beaver in Minnesota* and muskrats in British Columbia.** It is evidently widely distributed over the western plains of North America.

Histoplasma capsulatum, isolated first from man, was in 1947 discovered in rodents. Its distribution is centered about the east-central United States, particularly Virginia (10). In Fig. 6, *c* and *d* are photographs of a bone-marrow smear from a sternal puncture of an Alberta Indian woman who

* *Dr. A. B. Erickson, Minnesota Department of Conservation, St. Paul, Minn.*

** *Dr. I. McT. Cowan, Department of Zoology, University of British Columbia, Vancouver, B.C.*

FIG. 5. *Three genera compared. To the left, chlamydospores, to the right, conidia. a and b, Histoplasma capsulatum. c and d, Haplosporangium parvum. e and f, Blastomyces dermatitidis. Magnification: 1500.*

PLATE III

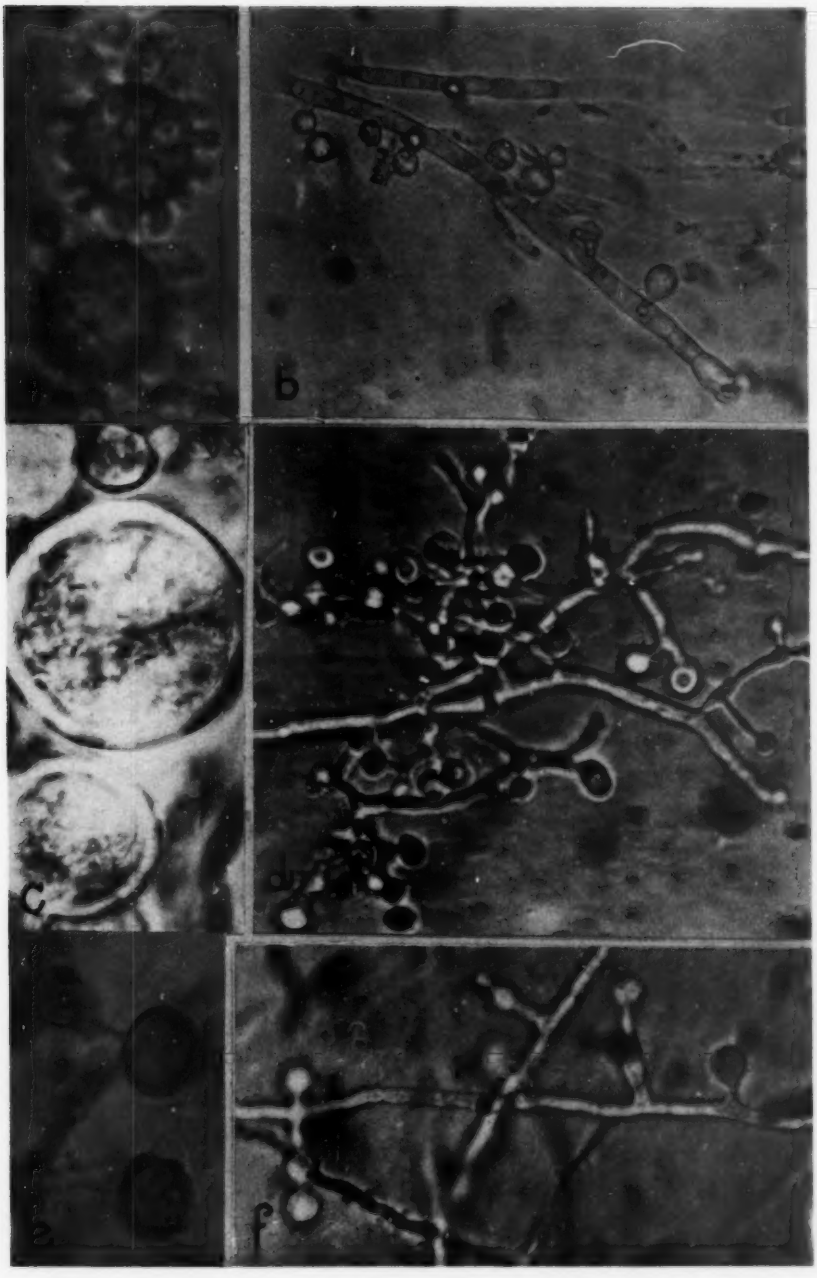
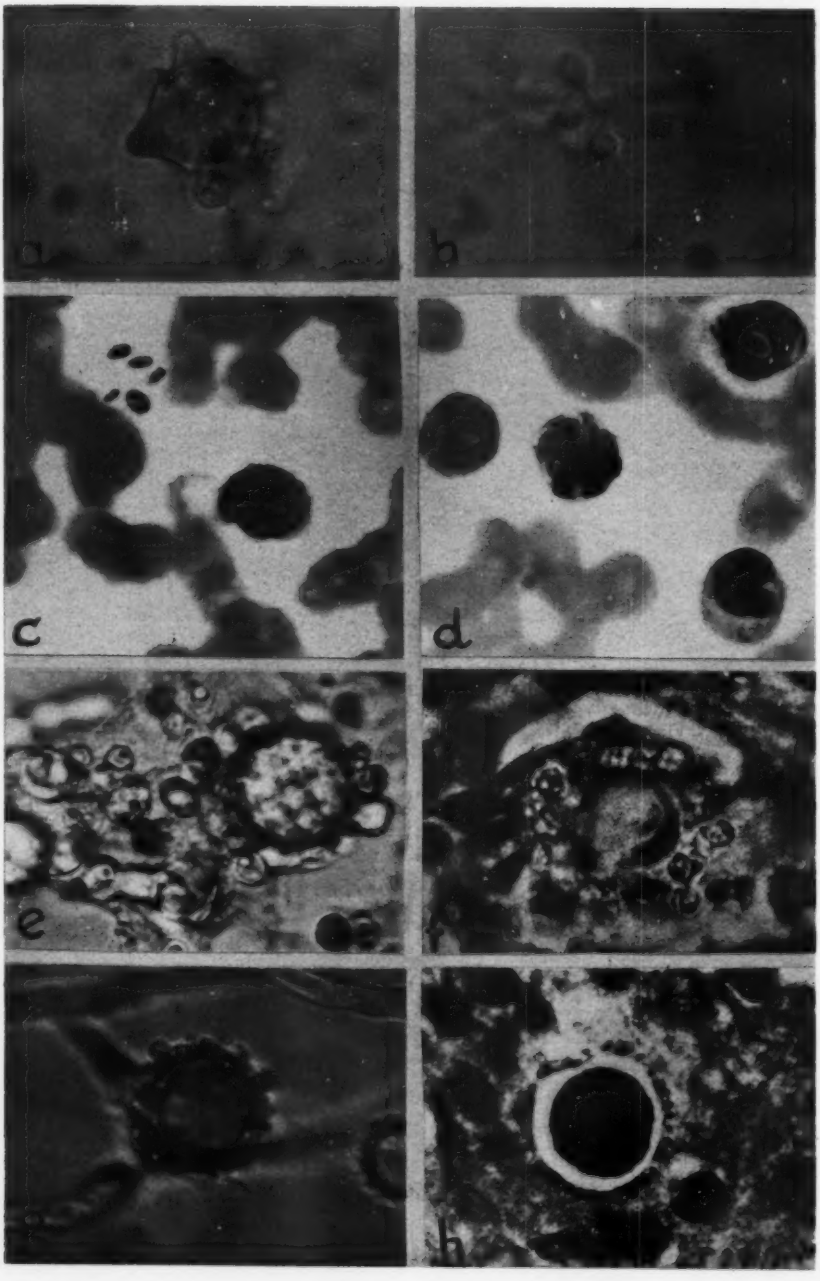


PLATE IV



subsequently died, but not from fungous infection. The smear was sent to Dr. N. F. Conant of Duke University who agreed with the author that it contained spores of *H. capsulatum*. If our conclusion is correct, *H. capsulatum* is also more widely distributed than may have previously been supposed.

Histoplasma and Blastomyces

It has previously been shown (7) that the spores of *H. capsulatum* resemble those of *B. dermatitidis* in their appearance and behavior. Fig. 5, *a*, *b*, *e* and *f*, shows their conidia and chlamydospores. The chlamydospores of *B. dermatitidis* differ from the tuberculate ones of *H. capsulatum*. Nevertheless as can be seen from an examination of the lower spore in Fig. 5, *e*, they are sometimes unevenly thickened, and may grow out into extensions from the thinner parts of the wall, recalling those of *Histoplasma*.

More remarkable is the resemblance between *H. capsulatum* and *B. brasiliensis*. The yeast cells of *B. brasiliensis* reproduce by multiple budding (4) (Fig. 6, *f* and *h*). The chlamydospores of *H. capsulatum*, in transition to yeast cells, also produce multiple buds. The multiple budding forms of *H. capsulatum* and *B. brasiliensis* are so similar that the two fungi cannot be distinguished on the basis of these spores.

Acknowledgments

This investigation was financed by the National Research Council of Canada. Dr. Harold Orr, Department of Dermatology, University of Alberta, again acted as sponsor and grantee. The author gratefully acknowledges the collaboration of her research assistant, Miss Anita Heckley.

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Fig. 6. *Histoplasma and Blastomyces. a to e, and also g, H. capsulatum; f and n, B. brasiliensis: a, chlamydospore with conidia; b, mycelium and conidia recovered from mouse that had inhaled spores; c and d, smear from sternal puncture of native Albertan (photographs by Dr. N. E. Conant); e, budding chlamydospore; f, budding chlamydospore from tissue of South American patient (after Fonseca and Almeida); g, as e; h, budding chlamydospore in liver from case of South American blastomycosis (after Conant).*

FAT ABSORPTION AND LUNG OIL[1]

By C. B. Weld[2]

Abstract

The lungs of normal animals and of normal animals fed with oil have been examined for the presence of oil. Frozen sections stained with Sudan IV and haematoxylin reveal Sudan stained intracellular granules and interstitial or intravascular oil globules in alveolar walls. In general these oil globules are larger and more numerous in the oil fed animals than in the normal controls. In the lungs of a considerable number of the animals absorbing fat, patchy areas of oil are found that resemble pulmonary fat embolism. Evidence is presented to show that the finding is neither a histological artefact, nor is it due to oil aspiration. Lung fat determinations give values higher in lungs showing marked oil deposits, but as a rule they remain within the normal range.

In the course of a study of the action of heparin on alimentary lipemia (8, 9) and of the possible use of heparin in pulmonary fat embolism (10), the observation was made that, in cats, relatively large oil globules, suggestive of pulmonary fat embolism, were present in the lung during alimentary lipemia.

In an attempt to obtain pulmonary fat embolism by *Clostridium Welchii* alpha toxin (10 and 2), a number of animals were injected with the toxin and lung specimens were taken. Occasional oil globules were found, possibly more than normal, but there was never a suggestion of clear-cut oil embolism. In the course of these experiments, the lung of a cat exhibiting alimentary lipemia was examined and was found to be loaded with oil. The globules were present interstitially, possibly in the capillaries, in the alveolar walls, and some in the alveolar spaces; they were patchily distributed, masses being present in some areas and none in other areas. The finding was so unexpected and seemed to have such little physiological reason that it was discounted. However, when the same finding (Figs. 1, 2, 3) was obtained in other animals it seemed necessary to accept it as a fact. The difference in appearance between the lungs from normal animals and the lipemic animals was striking. In the normal preparation, only an occasional small interstitial oil globule was seen. Further work was planned to confirm and extend the finding and to rule out the possibility of artefact.

Alimentary Lipemia

The animals were given oil by mouth. Cod liver oil or olive oil was trickled by syringe into the side of the mouth and was readily swallowed. An occasional animal exhibited some choking during the feeding; such animals were discarded. In general, dogs were given 20 cc. of oil or more, cats about 10 cc.,

[1] *Manuscript received December 8, 1947.*

Contribution from Department of Physiology, Dalhousie University, Halifax, N.S. Supported by a grant from the National Research Council.

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and guinea pigs about 5 cc. Larger doses of oil sometimes give clearer results but sometimes seem to delay digestion and prevent the production of lipemia. Specimens of lung and blood were taken usually two to seven hours later. When possible, blood was taken from time to time to follow the course of the lipemia before lung specimens were taken. Some animals were given sodium pentobarbital after being fed the oil, and in some, early lung biopsy was done in order to obtain successive lung specimens from the same animal. Occasionally good lipemia was obtained in animals injected with pentobarbital but never was it obtained after biopsy. One of the disheartening features of these experiments is the inconstancy of the lipemia, which varies greatly in time of appearance and in degree, often not appearing at all. It was planned to obtain specimens of lung systematically at various stages of lipemia and it was hoped that a lung biopsy before lipemia would give a standard of comparison but it has not been possible. However there are examples of a lung specimen taken at the height of lipemia and a second taken later when the lipemia had cleared, and there are also specimens of lung taken before oil was fed and again some hours later, though no lipemia occurred in the interval. In some of these specimens the difference in appearance is striking but not in all.

The blood specimens were oxalated, centrifuged in an angle centrifuge at about 4000 r.p.m. for 10 min., and the plasma appearance noted. Measurements of opacity were made with a Coleman Junior Spectrophotometer at a wave length of 680 mμ. Many plasmas showed a slight though distinct fogginess to reflected light but were clear to transmitted light. It has been suggested by Moreton (6) that this phenomenon may be due to very minute chylomicrons. The simple measure of light transmittance through the plasma serves very well as an index of the degree of lipemia in the more clear-cut examples, though it is not a completely satisfactory procedure in very minimal lipemias. Many of the plasmas were also examined microscopically, under the 40× objective with a 15× eyepiece and with dark field illumination, and a rough chylomicron estimation was made (5). The lung specimens for section were dropped into 5% formalin saline for 24 hr. or more. Frozen sections were cut, stained with Sudan IV and haematoxylin, and mounted in Farrent's solution. Chemical determination of total lipid was also done on many specimens. From several animals other tissues were also taken. In the liver there is a patchy fatty metamorphosis, oil droplets of variable size, both intracellular and interstitial, being present during the fat absorption. Other tissues have not yet been studied in detail.

It was thought that the conditions found were not caused by faulty histological technique, because standard methods had been used by competent workers; however the effect of variations in fixing and freezing has been examined. Satisfactory sections were not obtained from fresh lung but no difference could be seen between blocks fixed for about 18 hr. and those fixed

for a week or more. Blocks frozen and thawed several times also gave the same appearance as those frozen only once. As a further check on the possibility of the freezing causing a change in dispersion of the lipid, gelatin to the extent of 5% was added to lipemic plasma and sections were cut of this gel, at once and after fixation in 5% formol saline for varying periods. In all cases the appearance was alike and consisted of a very finely granular Sudan staining material. The suspension was somewhat coarser but similar in appearance to the chylomicrons of the original plasma, and there was no suggestion of formation of oil globules. A variant of procedure that did make a difference to the appearance of the sections of lung was to allow them to dry on the slide, after staining before mounting. Oil globules in these sections seemed to lose their refractility and to be somewhat absorbed by the surrounding tissue; they were fainter and more diffuse. Care was therefore taken to add Farrent's solution for mounting before the sections became dry.

From these observations therefore, it would seem that the presence of the oil globules in the tissues is real, and not due to histological artefacts.

The earlier consistent and striking difference between the lung appearance in the lipemic and fasting animals has not been maintained. Occasional oil globules are quite commonly seen in the normal controls and many of the lipemic animals failed to show a clear-cut increase in the number or size of oil globules. However it remains true that the only preparations showing masses of oil were from animals that had been fed oil. Of the 14 normal controls, six were rabbits, four were guinea pigs, two were cats and there was one dog and one monkey. All were included in the control group; there seemed to be no species difference in the number or size of oil globules. Two of the controls showed moderate oil while 12 showed minimal or none. Of the 38 test animals, 15 showed marked oil deposits, six moderate, and 17 minimal or none. The controls were not fed on the day of the experiment, but had been fed as usual

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Frozen sections stained with Sudan IV and haematoxylin.

The red staining oil globules show as black on the orthochromatic film, but other dense areas may also be very dark. Some of the definite red oil globules are marked with white crosses.

The magnifications given are those of the microscope objective and ocular that were used.

The total enlargement of the final printed figure is about one-half this.

FIG. 1. *Cat No. 17, 7 cc. of cod-liver oil by mouth, lung taken three hours later, mild lipemia. Lung, ×40 ×7.*

FIG. 2. *Dog No. 17, 60 cc. of cod-liver oil mixed with food, lung taken five hours later, marked lipemia. Lung, ×40 ×15.*

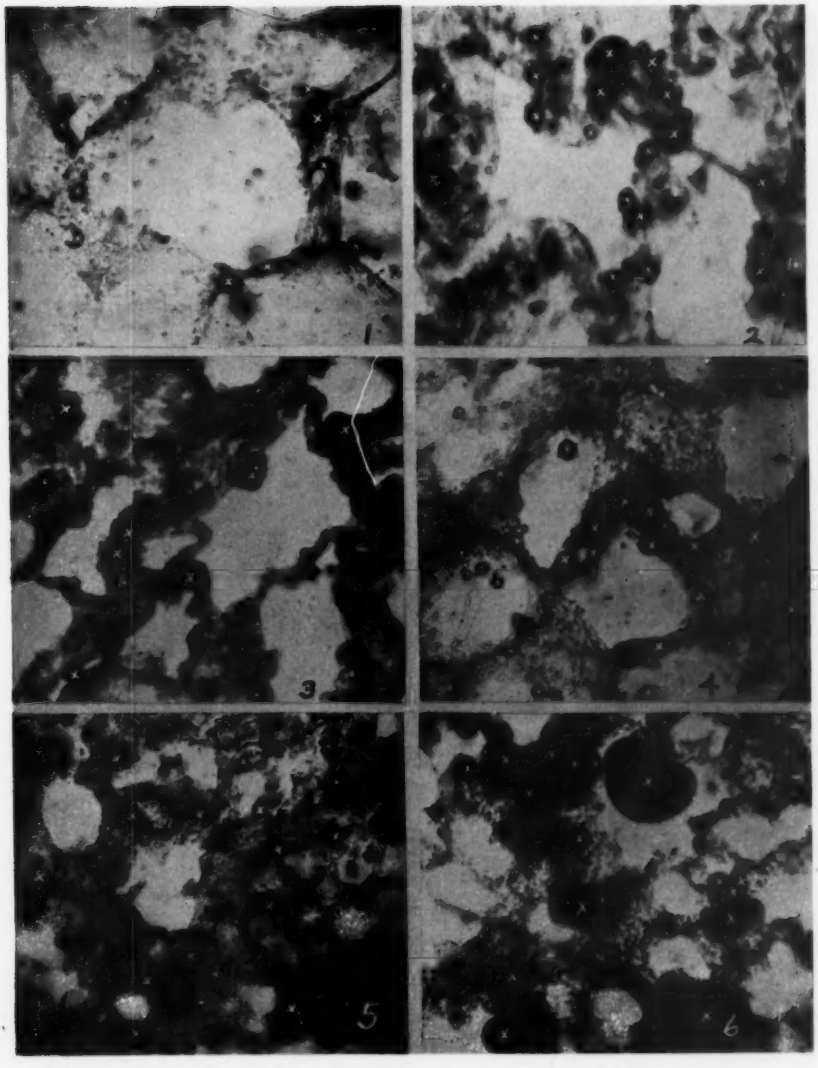
FIG. 3. *Cat No. 12, 10 cc. of cod-liver oil by mouth, lung taken six hours later, marked lipemia. Lung, ×40 ×7.*

FIG. 4. *Dog No. 8, 80 cc. of cod-liver oil injected intravenously at the rate of 2 cc. per minute. Lung taken at once. No lipemia. Lung, ×40 ×7.*

FIG. 5. *Guinea pig No. 15, 2 cc. of cod-liver oil by mouth, lung taken five hours later, marked lipemia. Lung, ×40 ×7.*

FIG. 6. *Guinea pig No. 28, 1.5 cc. of cod-liver oil by tracheal cannula, lung taken 15 min. later. Animal under sodium pentobarbital. Lung, ×10 ×7.*

PLATE I



on the previous morning. Contrasting the normal and test groups in the marked oil and minimal oil categories, the number of animals in each group is shown in Table I below.

TABLE I

NUMBER OF ANIMALS SHOWING OIL DEPOSITS IN LUNG

	Marked	Minimal
Normal, not fed	0	12
Oil fed	15	17

These results show a highly significant difference between the lung oil deposits of the normal and oil fed groups, the figures yielding a corrected Chi square of 6.6 (Yate's correction).

The results for all the animals, grouped in various ways, are shown in Table II.

TABLE II

NUMBER OF ANIMALS SHOWING OIL DEPOSITS IN LUNG

	Marked	Moderate	Minimal
Normal—fasting	0	2	12
Oil fed—all species	15	6	17
" —non lipemic, all species	5	2	6
" —lipemic, all species	10	4	11
" —cats	6	3	1
" —dogs	3	2	7
" —guinea pigs	5	1	8
" —rabbits	0	0	1
" —monkey	1	0	0

It will be noted from these figures that there is no difference in the oil fed group between the animals developing lipemia and those not developing lipemia. As between species, the cats seem to show greater oil deposits after feeding but the difference is hardly significant. Taking the group as a whole, however, it is evident that there is a definite positive relationship between oil feeding and interstitial oil globules in the lungs.

Furthermore, in many of the sections there were cells containing Sudan staining granules. They were particularly numerous just under the pleural surface. These intracellular lipid granules seemed to be more numerous in the oil fed animals and, in these, in a number of areas they appeared to be coalescing to form definite oil globules that then were extruded to form intracapillary globules. Whether or not some such mechanism is the source of many of the interstitial oil globules is purely speculative. Moreton (6) has

suggested that phagocytosis is stimulated by larger rather than by the smaller chylomicrons and has pointed out that in alimentary lipemia the chylomicrons become larger, a greater number thus becoming visible. On the other hand, the oil globules in lung appear in the animals fed fat whether or not they develop lipemia. According to Frazer (3) the lipemia is due to lymphatic fat absorption while active fatty acid absorption without a lipemia may occur by the portal route. The finding of excess lung oil in oil fed animals not exhibiting lipemia coupled with the marked fatty changes in the liver suggest that the liver may be the source of at least some of the oil globules found in the lung.

Perfusion Experiments

Because of the uncertainties involved in the production of alimentary lipemia and the practical difficulties in obtaining animals in a comparable degree and stage of lipemia, perfusion experiments were devised. It was thought that a lipemic plasma from one animal perfused through the lung of the same or another animal at a controlled rate would allow more uniform experimental conditions than was possible when simple feeding experiments were used. This was achieved only a few times. Difficulty was experienced in obtaining enough lipemic plasma from small animals, and the expedient of perfusing dog plasma through rabbit lung was tried. As the perfusion lasted only about one-half to one hour and the animals were not previously sensitized to the foreign protein, it was thought that it would be satisfactory. The animals were anesthetized with sodium pentobarbital, given artificial respiration by tracheal cannula and pump, and the thorax opened. Cannulae were rapidly inserted into the pulmonary artery and left auricle, and plasma (which usually showed a light transmittance of about 50%) run into the artery by a continuous injection pump at the rate of about 5 cc. per minute. The effluent from the left auricle was collected serially in centrifuge tubes. At the beginning of the perfusion there was often a slight reduction in the opacity of lipemic plasma during its passage through the lung, but in the main it passed through unchanged. After the perfusion the lungs showed no excess oil globules.

These experiments suggest that the lung does not take up any great amount of oil from lipemic plasma but they are not conclusive. The rate of the perfusion is probably very much less than the blood flow of the intact animal. The total volume of fluid perfused and its fat content may well be too small to allow a demonstrable amount of oil to collect in the lung, even if it were doing so.

To overcome this difficulty, an attempt was made to make an artificial oil emulsion, which could be made much more concentrated and in large quantities. Cod liver oil or olive oil emulsions were successfully prepared with a particle size of less than 1μ, and a transmittance of perhaps 1.0%. These emulsions were quite stable and kept well for days and, usually diluted to give a transmittance of about 10%, were perfused in a large number of rabbits and cats at the rate of 1 to 5 cc. per minute. They were prepared as follows,

the emulsifying agents being suggested by Frazer (4): 5 to 10 cc. oil, 2 to 3 cc. monobutyrin, 1 to 2 cc. oleic acid, 0.1 gm. sodium glycocholate, 3 to 5 gm. isinglass, and sometimes a trace of lecithin were made up to 100 cc. with Locke's solution, mixed, and brought to a pH of about 7.2. This was then passed through a hand homogenizer and centrifuged vigorously, the subnatant fluid being carefully drawn off and examined microscopically. If any globules larger than minute particles exhibiting Brownian movement were seen, the emulsion was recentrifuged. Some batches emulsified easily and others did not work at all. The protein was essential.

Even though the emulsion seemed stable and could be diluted freely with saline, the perfusion experiments with artificial emulsions were a failure. During passage through the lung the emulsion broke and the effluent was curdled. The lung showed masses of debris and oil globules, sometimes typical of pulmonary fat embolism. It was then found that the mere addition of blood or plasma to the artificial emulsion caused the emulsion to break. Attempts to use plasma instead of the isinglass in the original preparation of the emulsion proved unsuccessful and the method was dropped.

Lung Fat Determinations

Some of the lungs were not only examined histologically for oil but chemically as well. Portions of wet lung (1 to 2 gm.) were digested with sodium hydroxide over a steam bath. The digest was neutralized, extracted with ether and alcohol-ether, and the ether layer evaporated to dryness by radiant heat. The residuum was then extracted with petroleum ether, rinsed into weighed beakers, dried with radiant heat, and weighed. Duplicate samples checked within about 10%. The lipid content of the lungs of dogs, cats, and rabbits ranged from 11.0 mgm. to 22.4 mgm. per gm. of wet lung, the mean being 16.0 mgm. The standard error of the mean was ± 0.92. In guinea pigs the value was significantly higher, ranging from 20.5 to 29.8, averaging 24.4 mgm. (standard error ± 1.21). In these animals more Sudan staining intracellular granules were found than in the other species. In no species was there a significant relation between the degree of lipemia and the lipid value. Microscopically there was often a peribronchial accumulation of fat, probably adipose tissue, that was very variable and it was thought that this accounted for most of the lipid found. Specimens frankly loaded with oil gave high values, but, except when oil was aspirated, the values were no higher than in some normal specimens practically free of interstitial oil globules.

Aspirated Oil

The animals were not anesthetized and the oil was given by mouth; it was assumed that unless some choking occurred during the feeding of oil no aspiration of oil would have taken place.

It has been noted that some animals (guinea pigs) exhibited some choking when the oil was being given by mouth. It was stated that these animals were discarded. The lungs of five, however, were examined though the results have not been included in previous discussion. Three of these five animals showed considerable oil in large globules in the lung, most of the globules being in the alveolar spaces. This finding makes it necessary to consider the possibility that oil was aspirated by the animals that did not choke. Cannon (1), in a review of lipid pneumonia has emphasized the possibility that prolonged administration of oil, particularly by the nasal route but also by mouth, may result in aspiration oil pneumonia.

Two dogs were given their oil soaked into biscuits, and two cats were given theirs in a fish paste. In all four instances the food was eaten naturally and quickly, so it is extremely unlikely that any oil would be aspirated. The lungs of three of these animals showed interstitial oil globules but not definitely more than sometimes found in normal animals. The lung from one dog, however, showed many interstitial globules (Fig. 2), and many cells contained Sudan staining granules some of which seemed to be coalescing into globules that were extruding into the tissue spaces.

A number of animals were then given intratracheal oil. All were anesthetized with sodium pentobarbital and all were breathing naturally. A guinea pig had one lung removed as control and then 1.5 cc. cod liver oil was dropped into the tracheal cannula. Fifteen minutes later, in contrast to the clear control, the test lung contained masses of oil globules that filled many alveoli (Fig. 6). By analysis the lung contained nearly three times as much lipid as the control. Note that the photomicrograph was made with the low power objective; the intra-alveolar oil droplets are much larger than those in Fig. 5, which is also from a guinea pig. This animal had been given oil by mouth, without choking, and practically no oil droplets were found in the lung. However in one small area much oil was found, and as seen by the figure, it is intra-alveolar. Presumably, some aspiration had occurred. In this case the oil was given five hours before the lung was obtained while in the case of Fig. 6, there was only 15 min. between administration of oil and the taking of the lung. This time relationship probably accounts for the difference in appearance between Fig. 5 and Fig. 6, but the intra-alveolar oil in these contrasts sharply with the intravascular or interstitial globules in Figs. 1, 2, and 3.

A rabbit was then given 1 cc. of oil by tracheal cannula. Ten minutes later one lung was removed. This lung was clear of oil. The other lung taken after one and one-half hours was loaded with oil in the alveolar spaces, and its lipid content by analysis was more than three times that of the first. There was no lipemia. Two litter mate kittens were then used, one as control. The other was given two drops of oil by tracheal cannula and lungs were taken after one hour and three hours. Neither of these nor the control showed excess oil by microscope, nor did lipid analyses reveal any differences. Two rabbits and a guinea pig were then allowed to inhale an oil fog for about an

hour. The oil with 0.1% Aerosol T and an equal volume of water was nebulized and the fog directed into the side tube of a tracheal cannula. None of these three lungs showed excess oil.

From these experiments it seems clear that small amounts of oil can enter the trachea without reaching the alveoli. Large quantities of oil placed in the trachea do flood the lung, after some delay. When this does occur, however, the oil globules are primarily in the alveoli and the appearance of the specimens is quite different from that described in relation to alimentary fat absorption. In the latter cases the oil is chiefly intravascular or interstitial and the number of globules found in the alveoli is much less. In some areas the appearance resembles that of definite pulmonary fat embolism produced by intravenous injection of oil. Fig. 4 is lung from a dog into which 80 cc. of cod-liver oil was injected intravenously at a rate of 2 cc. per minute. It will be noted that large amounts of oil (Fig. 6) are found to be intravascular but many globules are also found in the alveoli; the appearance is similar to that described by Warren (7) as pulmonary fat embolism.

Though the above evidence lends strong probability to the belief that in our oil feeding experiments the appearance of the lung was not due to oil aspiration, it is not quite conclusive. The direct approach to the problem would be to ligate the oesophagus, or to block entry of the trachea by a tracheal cannula, and inject the oil directly into the stomach. It was thought necessary to try this method even though it was feared that the operative procedure would delay or even stop oil absorption.

Under sodium pentobarbital anesthesia the oesophagus of four cats was cut and both ends closed, the oil then being injected into the lower portion of the ligated oesophagus. A tracheal cannula was placed in one cat and two puppies and the oil given by stomach tube. Lung was taken two to five hours later. In only one animal was there a lipemia and in this animal the lung findings were indefinite. Three animals showed no signs of oil absorption (intestinal quiescence, invisible lymphatics, oil still in stomach at autopsy) and in these the lungs were negative for oil droplets. The other three animals showed some indication of alimentary activity (visible chyle in lymphatics, or intestinal movements, or little oil in stomach) and in all of these the lungs contained interstitial or intravascular oil globules of size and number not seen in the other animals.

Thus the lung findings discussed may occur when there can have been no aspiration of oil; therefore it is reasonable to conclude that they are not due to oil aspiration.

Conclusions

Frozen sections of lung from dogs, cats, guinea pigs, and monkeys stained with Sudan IV and haematoxylin sometimes reveal intracellular Sudan staining granules and interstitial or intravascular oil globules. These are more numerous in animals absorbing a fatty meal and in a considerable number of these, patchy areas of oil are found that resemble pulmonary fat embolism.

The appearance is not due to a histological artefact nor is it due to the aspiration of oil.

Lung fat determinations give values higher than the normal range only when excessive oil deposits are present. Guinea pigs give higher values than cats, dogs, and rabbits.

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THE EFFECT OF BARBITURATES AND OTHER SUBSTANCES ON MOTION SICKNESS IN DOGS[1]

By R. L. Noble[2]

Abstract

A large series of barbiturates and other substances have been tested for their ability to prevent motion sickness in dogs. Many compounds were found to possess this activity, which was not related to the anesthetic property of the compound. A number of barbiturates were found to be considerably more active than V-12, which was used as a standard. Pyridoxine, hyoscine, and streptomycin were inactive in the tests described. Bulbocapnine possessed half the potency of V-12 and showed an additive effect when the two substances were given together.

In a previous paper (Noble (2)) a detailed study of the effectiveness of various types of motion in inducing vomiting in dogs was reported. A method of assaying motion sickness preventives on dogs was also described (Noble (3)). In a search for an effective form of human therapy a large number of barbiturates and other substances were tested on dogs. Many of these proved to have the ability to prevent motion sickness to a greater or lesser extent and not cause any undesirable symptoms in the animal. Since the effect on motion sickness appears to be a specific property of the compound and not related to its hypnotic action it was essential to predetermine what dose would be tolerated by dogs without causing sleepiness, in-co-ordination, or anesthesia. Following this the substance was administered to susceptible animals that were then exposed to motion on a swing. Since the number of compounds to be tested was large, it has not been possible to determine potency in a quantitative manner except for particularly interesting substances. For practical reasons only the effects of oral administration has been studied. It should be stressed therefore that where compounds are listed as inactive the statement applies only to the dose that was used.

Methods

A colony of dogs susceptible to motion was established and swung in a mechanical swing at regular intervals as previously described (Noble (2)). The animals were fasted for 18 hr. before the test, the drug was administered in a small portion of minced meat two to four hours before swinging except in special cases where indicated. Immediately before being placed on the swing the animal was fed a moderate meal of meat. When dogs of different degrees

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of susceptibility were used, these were divided by their response to different intensities of motion as reported in a previous paper (Noble (2)). Vomiting was used as the only criterion of sickness but in some cases where the time taken to vomit was prolonged to 40 min. and much beyond the control time, the animals were listed as "improved" by treatment. Swinging was discontinued immediately after the animal vomited or after 45 min., in which case the animal was listed as protected. The barbituric acid derivatives were prepared and supplied through the co-operation of Abbott Laboratories and Eli Lilly and Co. Compounds supplied by the latter are designated by the letter A following the number. In initial tests each substance was given to groups of five to eight susceptible dogs. It was soon noted however that the effectiveness of therapy varied widely and was related to the degree of susceptibility to motion of the animal. Since it was possible to determine the susceptibility of the animals they were divided into three groups. In a second series of tests the susceptible dogs were selected at random but the dose of the compound was altered depending on whether the animal was of low, medium, or high susceptibility (Noble (2)). The group of dogs of medium susceptibility were considered as average and given the particular dose. The low susceptibility group received one-half this dose and the highly susceptible group, twice the dose. In the final series of tests the most satisfactory procedure was adopted so that groups of dogs of the same degree of susceptibility were selected and their response determined to one compound, V-12, which served as a standard. Other substances were then compared with V-12. The results of these three types of tests will be listed separately in results.

Results

Substances of No or Low Protective Action

For preliminary screening many substances were tested on two to four dogs (or more where indicated). If there was no evidence of protection in the dose used the substances were not considered worthy of further study. For reference purposes they are listed in Table I.

It is of interest to note that belladonna derivatives appear to be inactive in dogs in contrast to their protective action in humans. Similarly the vitamins, nicotinic acid and pyridoxine were also inactive.

Compounds Tested on Susceptible Dogs Selected at Random

A series of compounds were administered at various dose levels to groups of susceptible dogs. These have been listed in Table II in apparent order of activity although the results are of little quantitative value. The first figure in the table refers to the number of dogs protected against vomiting, the second to those that went 40 min. or more and then vomited but were considered improved, and the last figure to those unaffected by treatment.

TABLE I

SUBSTANCES INACTIVE IN PROTECTING AGAINST MOTION SICKNESS IN DOGS

Compound number	Substances*
Dose, 100 mgm./kgm.	
1	N-Methyl b.a.
3	C-C-dimethyl b.a.
19	Allyl-1-methylbutylacetylurea
1-A	Thiourea
8	*n*-Butyl-*n*-amyl b.a.
Dose, 30 mgm./kgm.	
7	Ethylacetoaminophenyl b.a.
11	*n*-Butylcyclohexyl b.a.
16	Diallyl-N-allyl b.a. (calcium salt)
28	Isopropylcyclohexenyl t.b.a.
37	Ethyllauryl t.b.a.
38	Ethyloctyl t.b.a.
39	Ethylheptyl t.b.a.
40	Ethyldecyl t.b.a.
42	Dibutyl t.b.a.
43	Allylbenzyl t.b.a.
49	Monobenzyl t.b.a.
61	Diphenylenehydantoin
63	Allylphenyl t.b.a.
65	*n*-Butylphenyl t.b.a.
69	*n*-Hexyl-*n*-propyl t.b.a.
77	Ethylphenyl t.b.a.
54	Mono-1-3-dimethylbutyl t.b.a.
26	Ethyl-1-methylbutylimino t.b.a.
Dose, 10 to 20 mgm./kgm.	
51	Ethylcyclohexenyl t.b.a.
72	Ethylisopropyl t.b.a.
73	Ethyl-2-ethylbutyl t.b.a.
86	Ethyl(methylamylcarbinyl) t.b.a.
11-A	*n*-Propylcyclopentenyl t.b.a. (sodium salt)
9	Ethyl-2-ethylbutyl b.a.
15	Allylbutylimino t.b.a.
2-A	Diphenylhydantoin
29	Allyl-1-methylbutylimino t.b.a.
47	Ethylcyclohexyl t.b.a.
Dose, 5 mgm./kgm.	
18	Ethyl-*sec*-butyl-N-methyl b.a. (calcium salt)
58	Ethyl(methylvinylcarbinyl) b.a.
59	Ethyl-2-pentenyl b.a.
10	Ethyl-1-methylbutyl-N-methyl b.a. (calcium salt)

* *Dosage of additional substances*
"Vasano" (hyoscine camphorate), 0.1 mgm.;
hyoscyamine camphorate, 0.4 mgm.;
nicotinic acid 25 mgm. and 50 mgm. (six tests);
pyridoxine 50 mgm. and 100 mgm. (eight tests).

Note: *b.a. = barbituric acid; t.b.a. = thiobarbituric acid.*

TABLE II

PROTECTIVE EFFECT OF BARBITURATES ON SUSCEPTIBLE DOGS

Compound number	Substance	Dose, mgm./kgm.			
		30	20	10	5
5	Allyl-*sec*-butyl b.a.			1–0–0	3–1–0*
9-A	Ethylcrotyl t.b.a. (sodium salt)			4–0–0	1–1–1
91	Isopropyl-1-methylallyl t.b.a.			3–0–0	0–1–0
104	Allylisoamyl b.a.		3–0–0	4–1–0	
44(V-12)	Ethyl-β-methylallyl t.b.a.		4–0–0	5–2–1	1–0–2
95	Ethyl-β-methylallyl t.b.a. (sodium salt)			1–1–0	1–0–3
100	Ethyl-β-methylallyl t.b.a. (calcium salt)				0–2–0
108	Allylcrotyl t.b.a.			2–2–0	0–3–0
64	Ethyl-1-methylallyl t.b.a.			4–1–3	
87	Allyl-1-methylisoamyl t.b.a.			3–4–0	
81	Allyl-1-methylbutyl t.b.a.		1–0–1	3–2–0	0–1–1
71	Allyl-*sec*-butyl t.b.a.			3–0–2	0–1–1
50	Allylisopropyl t.b.a.			2–2–3	
12	Ethylallyl t.b.a.	8–2–1		2–0–3	
23	Ethyl-*n*-butyl b.a.	3–0–0	4–0–0		0–0–2
24	Ethyl-1-methylbutyl b.a.	2–0–1	4–1–1		1–0–6
35	Ethyl-1-methylbutyl t.b.a.	3–0–0	5–0–1	0–0–2	
45	Ethyl(γ-chlor-β,γ-butenyl) t.b.a.	1–0–0	4–0–0	2–0–3	
55	Ethyl-2,4-dimethylpentyl b.a.		3–1–0	1–1–2	
33	Ethyl-*sec*-butyl-N-methyl b.a. (calcium salt)		3–0–1	0–2–2	
75	Ethyl-*n*-amyl t.b.a.		3–0–0	0–3–1	
36	Ethylisoamyl t.b.a.	6–1–1	4–0–1		
46	Ethylbutyl t.b.a.		3–0–2	2–2–0	
48	Methyl-*sec*-butyl t.b.a.		3–2–1		
105	Ethyl-2-ethylhexyl b.a.		3–1–2	1–1–2*	
4-A	Ethylphenyl b.a. (sodium salt)	1–0–1	1–0–1		
5-A	Diethyl b.a. (sodium salt)	1–0–2	2–0–2		
84	Allyl-*n*-amyl t.b.a.		3–0–3		
57	Diallyl-N-allyl b.a.		2–1–0		
62	*sec*-Butylcrotyl t.b.a.	1–1–2	2–0–0		
6	Ethylmethylhexylcarbinyl b.a. (sodium salt)	3–0–0	1–0–1		
56	Ethyl-*n*-hexyl t.b.a.	7–2–2			
93	Ethyl-*n*-hexyl t.b.a. (sodium salt)			1–1–1	0–0–3
93-B	Ethyl-*n*-hexyl t.b.a. (calcium salt)	0–1–4			
31	Diallyl t.b.a.	7–0–1			
7-A	*n*-Butyl-1-methylallyl t.b.a.	5–3–0		0–0–3	
67	Allyl-*n*-hexyl t.b.a.	2–3–1			
68	*n*-Hexyl-β-methylallyl t.b.a.	2–3–0			
32	Methyl-*sec*-butyl-N-methyl b.a. (sodium salt)	3–1–2			

* *Number of dogs under each dose divided to show number protected, improved, and negative.*

Note: *b.a. = barbituric acid; t.b.a. = thiobarbituric acid.*

TABLE II—*Concluded*

PROTECTIVE EFFECT OF BARBITURATES ON SUSCEPTIBLE DOGS—*Concluded*

Compound number	Substance	Dose, mgm./kgm.			
		30	20	10	5
21	Ethyl-β, β-dimethoxypropyl b.a.	1–1–4			
22	Ethyl-α, γ-dimethoxypropyl b.a.	0–3–0			
41	Ethylcyclopentyl t.b.a.		1–1–0		
52	Ethyl-*sec*-butyl t.b.a.			1–1–0	
74	Diethyl t.b.a.	1–1–0	0–1–1		
85	Ethyl(methylhexylcarbinyl) t.b.a.		1–0–0	0–1–1	
99	Allyl-*sec*-butyl b.a. (sodium salt)			1–1–0	
92	*n*-Propyl-1-methylallyl t.b.a.		2–3–2		
103	*n*-Propylcrotyl t.b.a.		1–1–2	0–1–3	
78	*sec*-Butylallylimino t.b.a.	0–0–1	0–1–6	1–0–3	
79	*sec*-Butyl-β-methylallylimino t.b.a.		0–0–3	0–0–1	
83	Allyl-2-ethylbutyl t.b.a.		0–0–1	3–1–1	
88	Allyl-*n*-butyl t.b.a.		0–1–1		
102	Ethylcrotyl t.b.a.				1–0–1
101	Ethylcrotyl t.b.a. (sodium salt)				1–1–0
		Dose per kgm.			
		100	90	80	50
53	Mono-1-methylbutyl t.b.a.				2–1–5
27	Ethylphenyl t.b.a.	3–0–0	0–0–2		
34	Ethyl-1-methylbutylacetamide	3–0–0		1–0–2	

* *Number of dogs under each dose divided to show number protected, improved, and negative.*
Note: *b.a.* = *barbituric acid; t.b.a.* = *thiobarbituric acid.*

It may be seen that the majority of these compounds exhibited the property of protecting dogs against motion sickness and not causing any unfavorable symptoms. Because of the wide variation in susceptibility of the animals and the small numbers used in each test, the results can only be considered as roughly quantitative. It is of interest to note that in some cases the sodium or calcium salts seemed of less activity than the acid. This and other observations indicate that the more insoluble acid forms of the barbiturates are the most effective in therapy.

Compounds Tested When the Dose was Adjusted According to Susceptibility of the Dogs to Motion

As previously mentioned a second series of tests were made in which the animals tested received varied doses of the drug depending on their previously determined susceptibility to motion. The dose that is listed in the table refers to that given to the dogs of moderate susceptibility, animals in the

group of low susceptibility would therefore receive half this dose and those of high susceptibility twice this dose. The substances are arranged in apparent order of activity in Table III.

TABLE III

EFFECT OF SUBSTANCES AT DOSE LEVELS PROPORTIONAL TO SUSCEPTIBILITY OF THE DOGS

Compound number	Substance	Dose, mgm./kgm.		
		10	5	3
125	Dicrotyl b.a.	4–1–1	5–2–1	3–2–1*
106	Allyl-β-methylallyl t.b.a.		3–3–0	2–4–4
104	Allylisoamyl b.a.	7–0–0	3–5–0	4–2–3
115	*n*-Propyl-β-methylallyl b.a.	5–1–0	3–2–2	
110	1-Methylallyl-β-methylallyl t.b.a.		3–2–2	
44	Ethyl-β-methylallyl t.b.a.		3–1–3	
124	Allyl-β-methylallyl b.a.	1–0–0	3–1–2	
107	Di-β-methylallyl t.b.a.		2–2–2	
109	Crotyl-β-methylallyl t.b.a.	1–1–0	2–2–2	
9-A	Ethylcrotyl t.b.a. (sodium salt)		3–0–3	
12	Ethylallyl t.b.a.		4–1–5	
117	*sec*-Butylcrotyl b.a.		3–0–3	
116	*sec*-Butyl-β-methylallyl b.a.		2–1–3	
113	Diallyl-N-methyl b.a.		2–0–3	
118	Ethyl-β-methylallyl b.a.	5–1–0	1–3–2	
126	Ethyl-isoamyl b.a.		2–2–7	4–1–5
24	Ethyl-1-methylbutyl b.a.		3–0–4	
122	Di-1-methylallyl t.b.a.		1–2–3	
7-A	*n*-Butyl-1-methylallyl t.b.a.		2–1–4	
5	Allyl-*sec*-butyl b.a.		1–1–4	
121	Dicrotyl t.b.a.	3–0–2	1–1–3	
123	Crotyl-1-methylallyl t.b.a.		2–0–4	
111	*n*-Propylallyl t.b.a.		1–1–5	
112	*n*-Propyl-β-methylallyl t.b.a.		1–0–5	
90	Ethyl-1-methylisoamyl t.b.a.	6–1–0		
64	Ethyl-1-methylallyl t.b.a.	5–0–2		
105	Ethyl-2-ethylhexyl b.a.	0–2–3		
114	Ethyl-β-methylallyl-N-methyl b.a.	2–1–2		
92	*n*-Propyl-1-methylallyl t.b.a.	4–0–3		
91	Isopropyl-1-methylallyl t.b.a.	3–0–0		
95	Ethyl-β-methylallyl t.b.a. (sodium salt)	1–1–0		
103	*n*-Propylcrotyl t.b.a.	0–1–2		
83	Allyl-2-ethylbutyl t.b.a.	1–0–3		
88	Allyl-*n*-butyl t.b.a.	0–0–3		
105	Ethyl-2-ethylhexyl b.a.	0–2–3		
60	5,5-Diphenyl-2-thiohydantoin		0–0–3	
119	3-Nitrophthalylurea	0–0–2	0–0–5	

* *Number of dogs under each dose divided to show number protected, improved, and negative.*
Note: *b.a.* = *barbituric acid; t.b.a.* = *thiobarbituric acid.*

Compounds Compared with V-12 as Standard

In the third series of tests only dogs of high or moderate susceptibility to motion have been used since these were found to give more consistent results than those of low susceptibility. Each dog was standardized against V-12(ethyl-β-methylallylthiobarbituric acid) so that other substances were compared with this substance. Table IV shows the results obtained using

a group of four to five dogs of the highest susceptibility to motion and Table V those with a group of six or seven dogs of moderate susceptibility. The average time of vomiting of those dogs that vomited is listed in parentheses.

TABLE IV

EFFECT OF SUBSTANCES COMPARED WITH V-12 ON SIX TO SEVEN DOGS OF MODERATE SUSCEPTIBILITY

Compound number	Substance	Dose, mgm./kgm.		
		10	5	2.5
		Percentage protected (and average time of vomiting)		
44 (V-12)	Ethyl-β-methylallyl t.b.a. (15 mgm./kgm. = 100%)	66 (41)	0 (18)	
137	Ethylmethylcyclopropylcarbinyl b.a.		80 (40)	60 (40)
21-A	Ethyl-1,3-dimethyl-1-butenyl b.a.			50 (28)
17-A	Ethyl-1,3-dimethylbutyl b.a.		83 (45)	42 (28)
90	Ethyl-1-methylisoamyl t.b.a.		60 (15)	
115	*n*-Propyl-β-methylallyl b.a.		55 (27)	0 (24)
22-A	*n*-Propyl-1-ethoxyethyl b.a.		40 (26)	
107	Di-β-methylallyl t.b.a.		42 (20)	
108	Allylcrotyl t.b.a.		42 (21)	
118	Ethyl-β-methylallyl b.a.		33 (22)	
133	Allyl-2-hexenyl b.a.		33 (36)	
134	Allyl-2-pentenyl b.a.		33 (12)	
26-A	Ethyl-2,4-dimethylpentyl b.a.		33 (15)	
28-A	Allyl-N-methylphenyl b.a.		20 (23)	
72	Ethylisopropyl t.b.a.		20 (30)	
75	Ethyl-*n*-amyl t.b.a.		20 (32)	
125	Dicrotyl b.a.	84 (32)	16 (28)	
116	*sec*-Butyl-β-methylallyl b.a.		15 (24)	
117	*sec*-Butylcrotyl b.a.			15 (28)
109	Crotyl-β-methylallyl t.b.a.		15 (28)	
123	Crotyl-1-methylallyl t.b.a.		15 (25)	
12	Ethylallyl t.b.a.	0 (25)		
126	Ethylisoamyl b.a.	100	0 (25)	
18-A	Isobutyl-1-methylallyl t.b.a.		0 (26)	
121	Dicrotyl t.b.a.		0 (21)	
103	*n*-Propylcrotyl t.b.a.		0 (28)	
124	Allyl-β-methylallyl b.a.		0 (32)	
104	Allylisoamyl b.a.		0 (28)	
135	Ethyl-4-pentenyl b.a.		0 (23)	
24-A	(1-Methylbutyl)ethylacetylthiourea		0 (16)	
14	Ethyl-1,3-dimethylbutyl t.b.a.			0 (24)
132	Ethyl-*n*-propylvinylcarbinyl b.a.			0 (26)
19-A	5,5-Dimethyl b.a.		0 (25)	
32-A	(1-Methylbutyl)ethylacetyl urea		0 (25)	

Note: *b.a. = barbituric acid; t.b.a. = thiobarbituric acid.*

TABLE V

EFFECT OF SUBSTANCES COMPARED WITH V-12 ON FOUR TO FIVE DOGS OF HIGHEST SUSCEPTIBILITY

Compound number	Substance	Percentage protected (and average time of vomiting)		
		Dose, mgm./kgm.		
		15	10	5
44 (V-12)	Ethyl-β-methylallyl t.b.a. (25 mgm./kgm. = 80%, 20 mgm./kgm. = 60%)	20 (12)	0 (16)	
137	Ethylmethylcyclopropylcarbinyl b.a.			100 (2.5 mgm./kgm. = 20%)
21-A	Ethyl-1-3-dimethyl-1-butenyl b.a.			(2.5 mgm./kgm. = 20%)
17-A	Ethyl-1-3-dimethylbutyl b.a.		100	25 (15)
130-X	Ethyl-*n*-butylvinylcarbinyl b.a.			25 (13)
131-X	Ethylmethylvinylcarbinyl b.a.		50 (13)	
28-A	Allyl-N-methylphenyl b.a.		50 (40)	
75	Ethyl-*n*-amyl t.b.a.		50 (35)	
22-A	*n*-Propyl-1-ethoxyethyl b.a.		50 (12)	
106	Allyl-β-methylallyl t.b.a.		40 (15)	
107	Di-β-methylallyl t.b.a.		40 (21)	
118	Ethyl-β-methylallyl b.a.	80 (40)	40 (21)	
115	*n*-Propyl-β-methylallyl b.a.		20 (26)	
109	Crotyl-β-methylallyl t.b.a.		20 (21)	
123	Crotyl-1-methylallyl t.b.a.		20 (14)	
124	Allyl-β-methylallyl b.a.		20 (30)	
27-A	Allylphenyl b.a.		20 (27)	
57	Diallyl-N-allyl b.a.		25 (29)	
70	Ethylisobutyl t.b.a.		25 (22)	
125	Dicrotyl b.a.	0 (29)		
12	Ethylallyl t.b.a.	0 (31)		
18-A	Isobutyl-1-methylallyl t.b.a.		0 (15)	
117	*sec*-Butylcrotyl b.a.		0 (21)	
121	Dicrotyl t.b.a.		0 (13)	
108	Allylcrotyl t.b.a.		0 (23)	
62	*sec*-Butylcrotyl t.b.a.		0 (18)	
54	Mono-1,3-dimethylbutyl b.a.		0 (14)	
136	5,5-Trimethylene b.a.		0 (12)	
30-A	Diphenyl b.a.		0 (10)	
26-A	Ethyl-2,4-dimethylpentyl b.a.		0 (19)	
23-A	Diethylmethylcarbinylurea	0 (16)		
25-A	*n*-Butyl-1-methylallyl acetamide	0 (21)		
24	Ethyl-1-methylbutyl b.a.			0 (22)
29-A	Phenylthienylhydantoin		0 (27)	
82	*n*-Butyl-*n*-propyl t.b.a.		0 (22)	
84	Allyl-*n*-amyl t.b.a.		0 (25)	
73	Ethyl-2-ethylbutyl t.b.a.		0 (14)	
83	Allyl-2-ethylbutyl t.b.a.		0 (13)	

Note: *b.a. = barbituric acid; t.b.a. = thiobarbituric acid.*

The results shown are considered to be the most accurate quantitative comparison of the more active compounds tested. These have been listed in terms of activity compared with V-12 in Table VI.

TABLE VI

ACTIVITY IN TERMS OF V-12

Compound number	Substance	Number of times potency of V-12	
		Highly susceptible dogs	Moderately susceptible dogs
137	Ethylmethylcyclopropylcarbinyl b.a.	6	4
21-A	Ethyl-1,3-dimethyl-1-butenyl b.a.	6	3½
17-A	Ethyl-1,3-dimethylbutyl b.a.	3	3
130-X	Ethyl-*n*-butylvinylcarbinyl b.a.	3	—
131-X	Ethylmethylvinylcarbinyl b.a.	1½–2	—
90	Ethyl-1-methylisoamyl t.b.a.	—	2
28-A	Allyl-N-methylphenyl b.a.	1½–2	1–1½
75	Ethyl-*n*-amyl t.b.a.	1½–2	1–1½
22-A	*n*-Propyl-1-ethoxyethyl b.a.	1½–2	1½–2
106	Allyl-β-methylallyl t.b.a.	1½–2	—
107	Di-β-methylallyl t.b.a.	1½–2	1½–2
108	Allylcrotyl t.b.a.	—	1½–2
118	Ethyl-β-methylallyl b.a.	1½–2	1–1½
115	*n*-Propyl-β-methylallyl b.a.	1–1½	2
109	Crotyl-β-methylallyl t.b.a.	1–1½	1–1½
123	Crotyl-1-methylallyl t.b.a.	1–1½	1–1½
124	Allyl-β-methylallyl b.a.	1–1½	1
133	Allyl-2-hexenyl b.a.	—	1–1½
134	Allyl-2-pentenyl b.a.	—	1–1½
26-A	Ethyl-2,4-dimethylpentyl b.a.	—	1–1½

Note: *b.a. = barbituric; t.b.a. = thiobarbituric acid.*

It may be seen that the activity of most compounds was of approximately the same order irrespective of the group of dogs in which they were tested. The first four compounds listed seemed to have considerably greater activity than the standard V-12.

Other Observations

Common Barbiturates

In an early experiment susceptible dogs were selected at random and tested with four of the more common barbiturates at intervals of one week. The dose used was the same in each case, 20 mgm. per kgm. body weight. The results were as follows:

Sodium neonal (ethyl-*n*-butylbarbiturate)	seven dogs protected
Sodium nembutal (ethyl-1-methylbutyl barbiturate)	five dogs protected
Sodium phenobarbital (ethyl-phenylbarbiturate)	four dogs protected
Sodium barbital (diethylbarbiturate)	three dogs protected

Duration of Action

Experiments have been conducted on eight different barbiturates in which the duration of protective action has been determined. In general it may be stated that the protection of an effective dose usually persists for 18 to 24 hr. at which time the animals are either immune or considerably

improved. Longer protection is not found unless the dose given is excessive. Using V-12, six dogs received the minimum effective dose (M.E.D.) to prevent vomiting and were swung 22 hr. later; 16% were protected. When the dose was increased to twice the M.E.D. 66% were protected after the same interval. The same number of animals received three times their M.E.D. and were swung 48 hr. later. In this case no dogs were protected.

Repeated Doses

If small doses of active barbiturates are given at short intervals their effectiveness does not appear to be enhanced but equal to the total dose given. Even when the doses are spaced at 10 hour intervals the same additive action is observed. Daily doses of V-12 did not give rise to cumulative effects in that noneffective doses, even though repeated daily for 10 days, did not lead to a protective action.

Mixtures

Since it seemed possible that the drug bulbocapnine, which had been shown to be effective in preventing motion sickness in a number of dogs (Babkin, Dworkin, and Schachter (1)), might act in a different manner than barbiturates, some tests with mixtures were made. These have been summarized in Table VII.

TABLE VII

EFFECT OF V-12 AND BULBOCAPNINE ON MOTION SICKNESS

Drug	Dose, mgm./kgm.	Protected, %	Dose, mgm./kgm.	Protected, %
Moderately susceptible dogs (five animals)				
V-12	5	0		
V-12	10	60		
Bulbocapnine	20	60		
Bulbocapnine	10			
Hyoscine	0.43 mgm. (total dose)	0		
Bulbocapnine	5	40		
V-12	5			
Highly susceptible dogs (two groups of four animals)				
V-12	15	0	15	0
V-12	20	—	20	75
Bulbocapnine	10	0	—	—
Bulbocapnine	15	0	15	0
Bulbocapnine	20	0	—	—
Bulbocapnine	10	50	10	25
V-12	15		10	
Bulbocapnine	15	50	10	50
V-12	10		15	
Bulbocapnine	15	75	15	100
V-12	15		15	

It may be seen that bulbocapnine is approximately of half the activity of V-12 when given alone. When the two drugs are given together, however, definite summation of activity occurs so that noneffective doses of each may combine to give protection.

Streptomycin

The effect of streptomycin on interference with vestibular function in humans suggested it might be effective in motion sickness. Two dogs each received a single injection of 550 mgm. of streptomycin* but no protection against motion was observed. Two other animals received 300 mgm. by injection daily for 16 days. These showed no signs of ataxia and were not protected against motion by such treatment.

Discussion

The results that have been presented are a summary of experiments on dogs to attempt to find substances of value for the treatment of motion sickness. A large number of barbiturates have been shown to possess this action and from comparative tests it is apparent that this property is independent of the hypnotic or anesthetic potency of the compound. Such substances are orally active and appear to have a prolonged action after a single dose. It is of interest that a number of substances appear to be of considerably greater activity than V-12, a compound that has been shown to be effective against motion sickness in humans (Noble (4)). On the other hand some substances such as pyridoxine and hyoscine, that are effective in some types of vomiting in humans were of no activity in the tests on dogs. Bulbocapnine showed approximately half the activity of V-12 but the two substances when given together showed an additive effect.

The large number of barbiturates that possess activity in preventing motion sickness makes it difficult to determine whether any particular chemical configuration is more effective than another. From the tables it may be seen that many thio compounds are active although with substances like Nos. 44 and 118 (Table VI) and 24 and 35 (Table II) there is little difference in activity whether the compound contains sulphur or not. The use of thio compounds was chiefly considered as a means of reducing the duration and degree of sedative action. Similarly the use of substances with longer unsaturated side chains and substituted methyl groups were particularly effective and many in overdosage were of a convulsant rather than depressant action. The highly active compound No. 137 had powerful anesthetic properties in animals. Further studies may disclose a compound of low hypnotic activity yet highly protective against motion that would be more suitable for human therapy. Thio compounds would be less desirable because of possible toxic actions.

* *The streptomycin was kindly supplied by Ayerst, McKenna, and Harrison, Montreal, Que.*

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